dant. Its small, delicious fruit is an important article of trade, under the name of "Garambulla."

As we approach the valley of Mexico, we come into a more fertile region, producing tropical fruits and other products indicating the rich luxuriance which we are to encounter after another day's journey to the south or east. The mountain flora of the vicinity of Mexico is of special interest and beauty. Here there are many species of salvia, oxalis, verbena, geranium, *Solanum*, etc. Terrestrial orchids are decidedly numerous, though scarcely abundant, and the instant that we penetrate to the warm and moist valleys, even quite near to the city, interesting and handsome arboreal species begin to appear. Arboreal ferns, tillandsias and other bromeliads are also numerous. In rich places among the rocks dahlias of various colors are common and abundant.

(*To be continued*)

THE NATURE AND FUNCTION OF THE PLANT OXIDASES

By Ernest D. Clark

(*Continued from March Torreya*)

Function of the Oxidases in the Plant

Physiology

It is evident from the preceding chapters that oxidizing enzymes are very widely distributed. Since enzymes generally seem to be produced by plants or animals for some definite purpose in the life of the organism, it was natural that speculation should arise regarding the function of the oxidizing enzymes. Their usefulness to the plant probably lies in their power to act as accelerators of the ordinary processes of oxidation as we shall see in a closer study of their function in the plant.

The oxidases, more especially peroxidase and occasionally oxygenase, are found in seeds and seem to bear some relation to the age of the seed, state of germination, etc. Brocq-Rousseu and Gain[30a] examined the seeds of species of plants from many different families. They used both guaiac tincture and guaiacol with the addition of hydrogen peroxide as tests for peroxidase or "peroxydiastase," as they called it. Peroxidase was present in nearly all seeds examined, the amount decreasing with their age; however, in kernels of corn they found peroxidase after the corn had been standing for over two hundred years. They further noted that oxygenase was rarely present in the seeds, and also that the strongest test for peroxidase was given by the embryo. Bialosuknia[31] made glycerine extracts of resting and germinating seeds, testing these extracts for oxidases with guaiac tincture, indophenol reagent, benzidin, etc. Peroxidase was present in the resting seeds and at all stages of germination, while oxygenase (direct oxidase) could not be detected in the seeds before the second day, after which it was always present. Deleano[32] also made a study of the germination of seeds, getting the same results as those obtained by Bialosuknia. The catalase increased rapidly and then disappeared along with the fat. He found further that reductase (reducing enzyme) was present and that it was localized in the protein part of the seed. Issajew[33] made a careful study of the oxidase of germinated barley, his results agreeing with those of the other investigators already noted. He found the same increase of oxidases after germination and confirmed the presence of the so-called reducing enzymes under these conditions.

In the study of oxidizing substances and enzymes in biological materials, it soon became apparent that in many cases there occurred reducing substances along with the oxidases, etc. Frequently these reducing substances were called enzymes and given special names, such as the "philothion" of Rey-Pail-

[30a] Brocq-Rousseu and Gain. Sur l'existence d'une peroxydiastase dans les graines seches. Compt. Rend. Acad. Sci. 145: 1297. 1907.

[31] Bialosuknia. Ueber Pflanzen-Fermente. Zts. Physiol. Chem. 58: 487. 1908.

[32] Deleano. Recherche chemique sur lar germination. Centralbl. f. Bakt., II. Abt. 24: 130. 1909.

[33] Issajew. Ueber die Malzoxydase. Zts. Physiol. Chem. 45: 331. 1905.

hade,[34] who in 1888, announced that in beer yeast he had found a substance which caused the evolution of hydrogen sulphide from sulphur, even in the cold. In the potato, egg-plant, etc., Kastle and Elvolve[35] found that there were substances which reduced nitrates to nitrites, the most favorable temperature for this action being from 40° to 50°; the action being retarded by acids and much increased by benzaldehyde and benzyl alcohol. Action is also completely checked by boiling, but the authors hesitated to say that this action is due to an enzyme; they classified this reducing substance with those compounds that are unstable and easily oxidized, and which reduce nitrates, but not in unlimited quantity. This statement might also be applied to the so-called reducing enzymes found by Irving and Hankinson[36] in the Gramineae. In the action of both yeast and bacteria, reducing substances probably play a part, since they are usually present.

We may say, then, that reducing substances are of common occurrence in plants, both in the higher and lower representatives. In many plant juices there occur reducing substances which, in the test for oxidases with the color reagents, gradually decolorize all the mixture except a zone near the surface of the liquid; this upper colored part being immediately bleached if the solution is thoroughly shaken, but it reappears upon standing. These reducing substances, as well as catalase, may act as a check upon the activity of peroxidase in the living cell, but after death or narcosis, the production of reducing substances is lessened and the oxidases develop pigments, *i. e.*, oxidize the chromogens to colored compounds. It seems doubtful that these reducing substances are enzymes, since we know that ordinary reducing substances resulting from metabolism are present in practically all animal and plant cells. Such substances

[34] Rey-Pailhade: (*a*) Nouvelle recherche physiologique sur la substance organique hydrogénant le soufre à froid. Compt. Rend. Acad. Sci. **107**: 430. 1888. (*b*) Sur une corps d'origine organique hydrogénant le soufre à froid. Compt. Rend. Acad. Sci. **106**: 1683. 1888.

[35] Kastle and Elvolve. The Reduction of Nitrates by Certain Plant Extracts, etc. Am. Chem. Jour. **31**: 606. 1904.

[36] Irving and Hankinson. The Presence of Nitrate Reducing Enzymes in Green Plants. Biochem. Jour. **3**: 87. 1908.

may be formed by photosynthesis and in the metabolism of the plant. Heffter[37] believed that the so-called reducing enzymes are not enzymes at all, but that the reducing action is due to the decomposition products of protein, especially those containing the SH group. This, however, is denied by Fränkel and Dimitz[38] who believe that the reducing power of cells is due to their unsaturated fatty substances.

It seems likely that the oxidizing ferments assist in carrying on the oxidative processes of respiration by increasing the rapidity of the combination of oxygen with the oxidizable substances in the plant. It has long been known that there are certain plants which at times develop a temperature above that of their surroundings, representatives of the Araceae showing this peculiarity in a striking manner. Hahn[39] investigated this phenomenon in *Arum maculatum*, the spadix of which is often from 20° to 27° C. warmer than the surrounding air. He used press-sap from the spadix of the plant and found that upon exposure to the air, the liquid rapidly became greenish black; so he concluded that an oxidizing enzyme (tyrosinase) was present. Hahn allowed the press-sap to remain at 25° for several days and at the end of that time the content of sugars, originally high, dropped to nothing, with accompanying loss of weight in the carbon dioxide evolved. This process could be entirely prevented by heating the press-sap to 60° for half an hour before allowing it to stand. Furthermore, the same process took place in an atmosphere of hydrogen; so Hahn thought he was dealing with a case of intra-molecular respiration carried on by oxidizing enzymes. Krause[40] noticed a similar elevated temperature with loss of dry weight [probably carbohydrates] in *Arum italicum* and Knoch[41] did so in the case of the flower of *Victoria Regia*

[37] Heffter. Die reduzierenden Bestandtheile der Zellen. Med. Naturwiss. Arch. 1: part 1, p. 15. 1907.

[38] Fränkel and Dimitz. Gewebatmung durch Intermediärekörper. Wiener klin. Wochensch. 1909: No. 51, p. 1777.

[39] Hahn. Chemische Vorgänge im zellfreien Gewebsaft von *Arum maculatum*. Ber. Chem. Gesell. 33: 3555. 1901.

[40] Krause. Ueber die Blütenwärme von *Arum Italicum*. Abhandl. Naturfor. Gesell. zu Halle, 1882, p. 16.

[41] Knoch. Untersuchungen über den Physiologie, etc., der Blüte von *Victoria Regia*. Diss. Marburg, 1897.

at the time of the opening of its petals. As we have seen, the many striking changes of color in plants after injury with the resulting exposure to the atmospheric oxygen, have long been subjects of investigation, but until recently such research was confined to studies of the enzymes involved, to the consequent neglect of the chromogens affected by these enzymes. In studying the rôle of the oxidases, if we were to consider only the enzymes, we should be neglecting the other half of the problem, for the chromogens occurring in plants are the sources of all the colorations and may very well act as oxygen carriers in the metabolism of the plant. Even in 1882 Reinke[42] interested himself in the substances in the plant which gave colored oxidation products under the influence of oxidases and of the air. The juice of the potato and of the white beet contained a chromogen which became dark upon standing in the air, but it was easily changed back to its original colorless state by reducing agents or by certain bacteria. He thought that the colorless condition of the chromogens in the living cell is due to accompanying reducing substances, or else that the cell is able to oxidize the chromogens through the colored state to a more highly oxidized colorless condition.

To show the distribution of these chromogens among plants this outline, adapted from Chodat,[43] is given (the changes being from colorless to that indicated):

Yellow, to green, then to blue—*Boletus spp.*

Red, violet and then black—many of the higher fungi, especially Agaricaceae; wheat seedlings, potatoes, apples, nuts, *Lathyrus niger*, secretions of certain ink-fish, etc.

Brown, then black—*Rhus succedana*, etc.

Violet-red—*Jacobinia spp.*[44]

Black—the higher fungi, especially *Hygrophorus spp.*; *Monotropa uniflora* and *Viburnum lantana.*

[42] Reinke. Ein Beitrag zur Kenntniss leicht oxidirbarer Verbindungen der Pflanzen-körpers. Zts. Physiol. Chem. **6**: 263. 1882.

[43] Chodat. Chapter on the "Oxydases" in Abderhalden's Handbuch der Biochem. Arbeitsmethoden, III, 2d part, p. 42 ff. 1910.

[44] Parkin. On a Brilliant Pigment Appearing after Injury in Species of Jacobinia Report Brit. Ass'n Advancem. Sci. 1904, p. 818.

Palladin[45] and his collaborators have taken up the question of the rôle of the chromogens and the oxidases in the respiration of the plant. They have followed out the general line of thought first conceived by Reinke. They have published many papers on the subject which cannot be abstracted here in detail, but a general outline of their results and conclusions will be given. In the anaerobic respiration of seeds, alcohol, acetone, and substances of aldehyde nature were obtained. Oxygenase increases with the growth of the part containing it. Both oxygenase and peroxidase are much increased by feeding the plant freely with sugars. The chromogens also increase under such circumstances. Palladin made a systematic search for the respiratory chromogens, and found they were very wide-spread and were generally red or brown when oxidized. To detect the chromogens he ground the plant material under water and thus obtained a light-colored solution to which he added peroxidase (from horseradish) and hydrogen peroxide; if the chromogen were present, it was soon oxidized and caused the solution to darken. In this manner he found that of seventy-one different plants examined, sixty-seven contained chromogens and that the parts with an active respiration like flowers, young shoots, etc., showed the greatest amount of respiratory chromogen. Chloroformed plants soon began to show coloration due to the oxidation of their chromogens. These chromogens seem to be derivatives of the cyclic series, and Palladin considered that they often occur in the form of glucosides, which, by the action of glucoside-splitting enzymes, are separated from the sugars and then take up oxygen by the aid of the oxidases, thus becoming colored. During the normal life of the plant there is a coördinated action of these hydrolytic, oxidizing, and reducing enzymes, which prevents oxidation of the chromogens, but during narcosis or after death,

[45] Palladin: (*a*) Die Atmungspigmente der Pflanzen. Zts. Physiol. Chem. **55**: 207. 1908. (*b*) Die Verbreitung der Atmungschromogene bei den Pflanzen. Ber. Bot. Gesell. **26a**: 378. 1908. (*c*) Ueber das Wesen der Pflanzenatmung. Bioch. Ztsch. **18**: 151. 1909. (*d*) Ueber die Bildung der Atmungschromogene in den Pflanzen. Ber. Bot. Gesell. **26a**: 389. 1908. (*e*) Die Arbeit der Atmungsenzyme der Pflanzen, etc. Zts. Physiol Chem. **47**: 407. 1906. (*f*) Ueber die Prochromogene der Pflanzen-Atmungschromogene. Ber. Bot. Gesell. **27**: 101. 1909.

the inter-relation of these enzymes is disturbed, with the result that the respiratory chromogens become evident by their color. The fact that these respiratory chromogens may take up oxygen and later give it up again under the influence of reducing substances, led Palladin to call the respiratory chromogens the "phyto-haematins" because he thought they were similar to the oxygen-carrying pigments of the blood of animals.

This work of Palladin and his students upon respiratory chromogens is a valuable contribution to our knowledge of the respiration of plants. His conception of the respiratory pigments as being cyclic compounds bound to the sugars in the form of glucosides which are insoluble, seems to be founded on fact. In the case of indigo-blue, according to Walther[46] and also in the case of many other pigments, the chromogen is held in the insoluble glucoside form, from which it is separated by the hydrolytic enzymes to give sugars, and then the oxidases attack the chromogen thus set free, imparting to it a definite color. In *Schenckia blumenaviana*, Molisch[47] found that the green plant became red upon treatment with chloroform vapor. This result he attributed to the action of an enzyme upon a chromogen in the plant. In certain of the Dipsacaceae, Miss Tammes[48] demonstrated the presence of a colorless chromogen dipsacan which, under the influence of oxidases, was changed to a blue pigment called dipsacotin by this investigator. Miss Wheldale[49] believes that the red colorations of certain leaves and flowers are caused by anthocyan, a pigment resulting from the coördinated action of oxidases and hydrolytic enzymes. She also considers that the color or lack of color in the offspring of such plants is due to the action of oxidases and reducing substances, etc., as factors in heredity. Overton[50] and also Tswett[51] came to the con-

[46] Walther. Zur Frage der Indigo-bildung. Ber. Bot. Gesell. **27**: 101. 1909.

[47] Molisch. Ueber ein neues, einen karminroten Farbstoffe erzeugendes Chromogen bei *Schenckia blumenaviana*. Ber. Bot. Gesell. **19**: 149. 1901.

[48] Miss Tammes. Dipsacan und Dipsacotin, ein neues chromogen und neues Farbstoffe der Dipsaceae. Recueil. Trav. Bot. Néerland. **5**: 51. 1908.

[49] Miss Wheldale. Plant Oxydases and Chemical Relationships of Color Varieties. Prog. Rei. Botan. **3**: 457. 1910.

[50] Overton. Beobachtungen und Versuche über das Auftreten von rothem Zellsaft bei Pflanzen. Jahrb. Wiss. Botan. **33**: 171. 1899.

[51] Tswett. Ueber den Pigmente der Herbstlich-vergilbten Laubes. Ber. Bot. Gesell. **26a**: 98. 1908.

clusion that the beautiful autumn colors of leaves are due to this same process, when the slowing up of the metabolic processes of the plant by the frost tends to hasten the formation of the oxidized pigments. It should be noted that in many cases the tannins act in this manner when oxidized, after being set free from their glucoside form. In a very recent study of the rôle of the glucosides in the plant, Weevers[52] concludes that these substances may be considered as reserve foods, the cyclic compounds being attached to glucose-yielding substances of low diffusibility, thus serving to accumulate sugar, etc., for future use.

Besides this coördinated action of the hydrolytic and oxidizing enzymes just described, there also seems to be an antagonistic action between the oxidases and the reducing substances in the cell; this antagonism tending to keep each sort from getting the upper hand during life, but after death when the production of reducing substances ceases for a time, the oxidases run riot, and blackening as well as colorations of various sorts result. The blackening of the foliage of many plants after a frost, and the production of the red and gold of our autumn forests, are doubtless due to the killing of the leaves or to an interference with their metabolism by the low temperature, and consequent excessive activity of the oxidases upon tannins and other substances.

Finally, Czapek[53] has brought to light a most interesting example of the part played by oxidases in the life of the plant. He found that geotropically and phototropically stimulated plant organs always contained more reducing substances and also showed weaker tests for oxidases than similar organs unstimulated. Later he proved that the reducing substance which accumulated after stimulation was homogentisic acid, and that, after stimulation, it did not seem to be destroyed by the oxidases as it had been before. What caused this accumulation of easily

[52] Weevers. Die physiologische Bedeutung einiger Glycoside. Recueil. Trav. Bot. Néerland. **7**: 1. 1910.

[53] Czapek: (*a*) Ueber einen Befund an geotropsich gereizten Wurzeln. Ber. Bot. Gesell. **15**: 516. 1897. (*b*) Stoffwechselprocesse in der geotropisch gereizten Wurzelspitze, etc. Ber. Bot. Gesell. **20**: 464. 1902.

oxidizable substances in the stimulated plant parts? By a series of careful experiments Czapek demonstrated that there was no decrease in the *amount* of oxidases present, but that they were inhibited by some influence, this influence later proving to be an anti-enzyme. He showed that the anti-enzyme thus formed really neutralized the oxidizing enzyme in definite proportion; that it was specific for that one plant, less so for the genus and not at all for distantly related plants; that heating a mixture of anti-enzyme and enzyme to 62° destroyed the former, the latter then regaining its original activity. Czapek demonstrated also that the anti-enzyme does not exist at all in unstimulated parts of the same plants, but later is produced in them upon stimulation. This anti-enzyme has the power of inhibiting the normal oxidation of the homogentisic acid in the plant, so that after stimulation, both the homogentisic acid and the anti-enzyme make their appearance and accumulate. However, Graefe and Linsbauer[54] report that they were unable to find the increase of reducing substances in stimulated parts as claimed by Czapek.

LABORATORY OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY,
COLLEGE OF PHYSICIANS AND SURGEONS,
NEW YORK.

(*To be continued*)

CHONDROPHORA VIRGATA IN WEST FLORIDA

ROLAND M. HARPER

Ninety-three years ago that sagacious botanist, Thomas Nuttall, proposed as a new species *Chrysocoma virgata*,* describing it at some length, and remarking that it was allied to *C. nudata* Mx., but might easily be confounded with *Solidago tenuifolia*. The locality given for it was "On the borders of swamps in New Jersey, near the sea-coast." In 1836 A. P. DeCandolle included this species and a few others in his new genus *Bigelowia*,† and cited a specimen collected "in Florida prope Savannah."

[54] Graefe and Linsbauer. Zur Kenntniss der Stoffwechseländerungen bei geotropischer Reizung. Sitzber. Wien. Akad. I. Abt. **118**: 907. 1909.

* Gen. 2: 137. 1818.

† Prodr. **5**: 329. 1836.